IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellants:	P.K. Gupta et al.	Attorney Docket No.: 24866A
Application No.:	10/636081	Art Unit: 1661 / Confirmation No.: 9824
Filed:	August 6, 2003	Examiner: A.H. Para
Title:	METHODS FOR PRODUCING CONIFER SOMATIC EMBRYOS	

APPELLANTS' APPEAL BRIEF

Seattle, Washington
October 18, 2010

TO THE COMMISSIONER FOR PATENTS:

This brief is in support of a Notice of Appeal filed in the above-identified application on August 18, 2010, to the Board of Patent Appeals and Interferences appealing the decision dated May 19, 2010.

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I. REAL PARTY IN INTEREST

Weyerhaeuser NR Company, a wholly owned subsidiary of Weyerhaeuser Company, having a place of business at 33663 Weyerhaeuser Way South, Federal Way, Washington 98063, is the assignee of the entire interest of the appealed subject matter.

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II. RELATED APPEALS AND INTERFERENCES

None.

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III. STATUS OF CLAIMS

Claims 1-13, 17-19, 21, and 23-26 are pending in the application. Claims 14-16, 20, and 22 have been canceled. Claims 1-13, 17-19, 21, and 23-26 stand rejected under 35 U.S.C. § 103(a). Appellants now appeal the rejection of Claims 1-13, 17-19, 21, and 23-26. The table below indicates the status of all claims.

Claim(s)	Status	Appealed
1-13	Rejected	Yes
14-16	Canceled	No
17-19	Rejected	Yes
20	Canceled	No
21	Rejected	Yes
22	Canceled	No
23-26	Rejected	Yes

IV. STATUS OF AMENDMENTS

The application was finally rejected in a paper dated May 19, 2010. No amendments to the claims were filed subsequent to the final rejection. A copy of the appealed claims is attached in the Claims Appendix.

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V. SUMMARY OF CLAIMED SUBJECT MATTER

There is one independent claim on appeal, Claim 1. Claim 1 is directed to a method for producing a synchronized population of pine somatic embryos. (Specification, page 2, lines 12-13; and page 4, lines 1-2.) The method comprises:

(a) cultivating pre-cotyledonary pine embryogenic cells in, or on a maintenance medium comprising nutrients that sustain the pine embryogenic cells (Specification, page 8, lines 1-5; page 4, lines 7-10; and page 9, lines 8-9);

(b) cultivating pre-cotyledonary pine embryogenic cells from step (a) for a period from one week to two weeks in, or on, a synchronization medium that comprises maltose as the principal metabolizable sugar source, an absorbent composition and at least one synchronization agent selected from the group consisting of abscisic acid and a gibberellin, wherein the absorbent composition and the at least one synchronization agent are present at a concentration effective to produce a synchronized population of pre-cotyledonary pine somatic embryos wherein at least 50% of the pre-cotyledonary pine somatic embryos in the synchronized population are at the same developmental stage (Specification, page 2, lines 13-18 and 28-31; page 4, lines 2-7; page 4, line 29 to page 5, line 2; page 5, lines 6-7; page 6, lines 25-28; page 8, lines 5-11; page 9, lines 21-25; and page 11, lines 7-9); and

(c) transferring the synchronized population of pre-cotyledonary pine somatic embryos from step (b) to a development medium and culturing the pre-cotyledonary pine somatic embryos for a period from 9 to 14 weeks to produce a synchronized population of cotyledonary pine somatic embryos (Specification, page 7, lines 7-13 and 28-29; page 8, lines 11-13; and page 10, lines 9-11).

The term "pine embryogenic cells" is defined in the specification as any cells, including cells that are organized to form a tissue or an organ, derived from a plant of the order Coniferales

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(genus Pinus), that are capable of producing one or more conifer somatic embryos. (Specification, page 3, lines 23-25, and page 4, lines 7-10). The term "cotyledonary embryo" refers to an embryo that possesses at least one cotyledon. (Specification, page 3, lines 28-29). The term "pre-cotyledonary embryo" refers to an embryo that does not possess any cotyledons. (Specification, page 3, lines 29-30).

A "synchronized population" refers to a population of pre-cotyledonary embryos or cotyledonary embryos that are at the same stage of development; i.e., are generally uniform in stage, shape, size or quality. (Specification, page 4, lines 18-28).

The present invention produces a synchronized population of pre-cotyledonary embryos that progress through successive developmental stages together to yield a synchronized population of mature somatic embryos that can be germinated to form plants. (Specification, page 4, lines 25-28).

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VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Claims 1-13, 17-19, 21, and 23-26 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Pullman et al., U.S. Patent No. 5,294,549 (1994) in view of Gupta, U.S. Patent No. 5,563,061 (1996).

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VII. ARGUMENT

A. Claims 1-13, 17-19, 21, and 23-26 are Patentable Under 35 U.S.C. § 103(a) Over Pullman et al., U.S. Patent No. 5,294,549 (1994) in View of Gupta, U.S. Patent No. 5,563,061 (1996).

1. Claim 1

Claim 1 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Pullman et al., U.S. Patent No. 5,294,549 (1994) ("Pullman") in view of Gupta, U.S. Patent No. 5,563,061 (1996) ("Gupta"). See Examiner's Actions mailed May 19, 2010, and July 19, 2010.

It is the position of the Examiner that Pullman teaches cultivating pro-cotyledonary embryos in a maintenance medium; transferring the pro-cotyledonary embryos to a singulation medium comprising gibberellin and/or abscisic acid and activated charcoal; and transferring the pro-cotyledonary embryos to a development medium. The Examiner cites Gupta as teaching a singulation medium comprising maltose as the principal sugar source. In the view of the Examiner, it would have been obvious to one of ordinary skill in the art to use maltose as the principal sugar source in the singulation medium described in Pullman, in view of the teachings of Gupta.

It is further the view of the Examiner that by using known media and other well-known medium additives, it would be obvious that one skilled in the art would have obtained 50% or 75% of the embryo population at the same developmental stage, as claimed. See Office Action dated May 19, 2010.

As set forth below, appellants respectfully submit that the burden of establishing a *prima facie* case of obviousness of Claim 1 over the Pullman reference in view of the Gupta reference has not been met for at least the reasons that (a) the Examiner has not considered the teachings of

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the Pullman and Gupta references as a whole; (b) the Pullman and Gupta references, either alone or in combination, do not teach or suggest all the elements of Claim 1; (c) there is no motivation to modify the Pullman and Gupta references to arrive at the claimed invention; and (d) the claimed invention achieves unexpected results. Accordingly, Claim 1 is non-obvious and patentable over the Pullman reference in view of the Gupta reference.

a. Graham Factor Analysis

KSR confirmed that the Graham Factor Analysis should be used in determining whether a claimed invention is obvious under Section 103(a). *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1739 (2007). The Graham analysis includes assessing the rejected claims, the scope and content of the cited art, and the differences between the rejected claims and the cited art. *Id.* at 1734. The following subsections set forth the scope of Claim 1, the differences between Claim 1 and the cited references, and an explanation as to why the claimed invention is not rendered obvious in view of the cited references.

i. The Scope of Claim 1

Claim 1 is directed to a method for producing a synchronized population of pine somatic embryos, the method comprising:

(a) cultivating pre-cotyledonary pine embryogenic cells in, or on a maintenance medium comprising nutrients that sustain the pine embryogenic cells;

(b) cultivating pre-cotyledonary pine embryogenic cells from step (a) for a period from one week to two weeks in, or on, a synchronization medium that comprises maltose as the principal metabolizable sugar source, an absorbent composition and at least one synchronization agent selected from the group consisting of abscisic acid and a gibberellin, wherein the absorbent composition and the at least one synchronization agent are present at a concentration effective to produce a synchronized population of pre-cotyledonary pine somatic embryos wherein at least

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50% of the pre-cotyledonary pine somatic embryos in the synchronized population are at the same developmental stage; and

(c) transferring the synchronized population of pre-cotyledonary pine somatic embryos from step (b) to a development medium and culturing the pre-cotyledonary pine somatic embryos for a period from 9 to 14 weeks to produce a synchronized population of cotyledonary pine somatic embryos.

Somatic embryogenesis is a multi-stage process in which, generally, plant tissue is cultured in an initiation medium that initiates formation of embryogenic cells; the embryogenic cells are further cultured in a maintenance medium that promotes multiplication of the embryogenic cells; and the multiplied embryogenic cells are then cultured in a development medium that promotes development of somatic embryos, which are then germinated to form seedlings. See, e.g., Specification, page 1, lines 14-24.

A problem encountered in somatic embryogenesis is the asynchronous development of somatic embryos from cultures of embryogenic cells. The specification of the present application describes the problem as set forth below:

> A particular problem affecting conifer somatic embryogenesis is the asynchronous development of somatic embryos from cultures of embryogenic cells. This asynchrony in development results in cultures in which embryos are at disparate stages of development, greatly reducing the overall efficiency of the process.

Page 2, lines 2-5.

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> Cleavage polyembryony (embryonal suspensor mass proliferation) continues in cultures after plating onto development medium, and new embryos are beginning to develop even after eight to ten weeks of culture on development medium. Due to this continuing cleavage, embryos are not uniform in stage, shape, size, or quality within a single plate. This lack of uniformity detrimentally affects the efficiency of somatic cloning of conifers.

Page 4, lines 14-19.

The claimed method is directed to producing a <u>synchronized</u> population of pre-cotyledonary and cotyledonary <u>pine</u> somatic embryos. During examination, the USPTO must give claims their broadest reasonable interpretation in light of the specification, and words of the claims must be given their plain meaning unless the plain meaning is inconsistent with the specification. See M.P.E.P., Section 2111.01(I). It is evident from the above cited passages of the specification that a "synchronized population," as recited in Claim 1, is a population of pre-cotyledonary embryos or cotyledonary embryos that are at the same stage of development and are relatively uniform in size, shape, and quality.

The claimed method produces a synchronized population of cotyledonary pine somatic embryos by <u>adding a new culturing step</u> to the somatic embryogenesis process for <u>pine</u>, between the maintenance culturing step and the development culturing step. By culturing pre-cotyledonary pine embryogenic cells on a synchronization medium for a period of one week to two weeks, <u>after</u> culture on a maintenance medium and <u>before</u> culture on a development medium, the claimed method produces a synchronized population of pre-cotyledonary pine embryos that are at the same stage of development. The synchronized population of pre-cotyledonary pine embryos is subsequently transferred to a development medium and the pine embryos progress through successive developmental stages together to yield a synchronized population of mature somatic pine embryos that can be germinated to form plants. See Specification, page 4, lines 25-28.

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ii. <u>The Differences Between the Pullman Reference and Claim 1</u>

The Pullman reference describes a multi-stage conifer somatic embryogenesis process, which generally includes the steps of: (1) culturing explants on an induction medium; (2) culturing early stage proembryos from step 1 on a maintenance medium for multiplication; (3) culturing early stage proembryos multiplied in step 2 on a medium to develop late-stage proembryos; and (4) culturing late stage proembryos from step 3 on a development medium to develop cotyledonary embryos. See Col. 6, lines 22-24 and 29-34, and Col. 8, lines 11-12.

The Pullman reference describes different media compositions for use at various stages of the process and for different species. See, e.g. Col. 13 to Col. 14, Tables 1 and 2 (media compositions for culture of Douglas-fir); and Col. 20 to Col. 21, Tables 7 and 8 (media compositions for culture of Norway spruce). The Pullman reference makes it clear that the media requirements at different stages of the embryogenesis process are different for different species, as stated below:

> Since each plant species appears to possess a unique optimal set of media requirements, the successful preparation and regeneration of a new species <u>cannot be necessarily inferred</u> from the successful regimens applied to unrelated plant species.

Col. 2, lines 52-56 (emphasis added).

This difference in media requirements is also evident, for example, in the late proembryo development media composition, as described by Pullman:

> [F]or many species such as *Pinus taeda* and *Pseudotsuga menziesil*, the late proembryo development media should have a concentration of osmoticants that is significantly raised above that of the induction or multiplication media. The optimum osmoticant levels at each stage will usually <u>differ for each species and often for individual genotypes within a species</u>. For loblolly pine the osmotic level should typically be of the magnitude of at least 200 mM/kg and preferable about 240 mM/kg or even higher. However, lower levels of about 170 mM/kg minimum will suffice for most genotypes of Douglas-fir. . . . Some species such

> as *Picea abies*, which are relatively easy to reproduce, may not require this raised osmotic level, or it may only be necessary for some genotypes. In these cases late proembryo development may usually be achieved without a change in medium composition from the maintenance and multiplication medium.

Col. 7, line 52, to Col. 8, line 3.

In addition to the steps in the embryogenesis process described above, the Pullman reference describes an intermediate "singulation step" between the late proembryo development stage and the development stage that is required for the culture of Douglas–fir. See Col. 8, lines 18-21. Douglas-fir proembryos tend to form in tight clumps or clusters, which must first be singulated before transfer to the development media; i.e. the tight clumps or clusters must be separated into individual embryos, otherwise they will develop into a tight clump of cotyledonary embryos which cannot be readily separated and are difficult to use for further germination. See Col. 8, lines 18-23 and 45-48. However, Pullman specifically teaches that this intermediate step is "not necessary for other species." See Col. 8, lines 18-21 (emphasis added).

Moreover, in sharp contrast to the claimed invention, the Pullman reference does not teach or remotely suggest culturing pre-cotyledonary pine embryogenic cells on a synchronization medium comprising an absorbent composition and at least one synchronization agent (abscisic acid and/or gibberellin) before transferring the pre-cotyledonary pine embryos to the cotyledonary development medium, as claimed.

iii. The Differences Between the Gupta Reference and Claim 1

The Gupta reference describes a multi-stage conifer somatic embryogenesis process, which generally includes the steps of (1) culturing explants on an induction medium; (2) culturing early stage embryos from step 1 on a maintenance medium for multiplication; (3) further culturing early stage embryos on maintenance medium to produce advanced early stage embryos; and (4) culturing advanced early stage embryos from step 3 on a development

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medium to develop cotyledonary embryos. See Col. 6, lines 3-7; Col. 7, lines 21-23 and 58-59; and Col. 8, lines 42-44.

Similar to Pullman, Gupta describes different media compositions for use at various stages of the process and for different species. See, e.g., Col. 13 to Col. 14, Tables 1 and 2 (media compositions for the culture of Douglas-fir); Col. 21 to Col. 22, Tables 11 and 12 (media compositions for the culture of Norway spruce); and Col. 23 to Col. 24, Tables 13 and 14 (media compositions for the culture of Loblolly pine).

Gupta also describes an intermediate culturing step between the advanced early stage embryo growth stage and the cotyledonary embryo development stage for Douglas-fir to singulate the tight clumps or clusters of early stage embryos. See Col. 7, line 66, to Col. 8, line 4. Gupta explains, "[T]he terms 'singulation' or 'singulation stage' are fully equivalent to 'maintenance culture' or 'maintenance stage.' The singulation stage may be considered a specialized type of maintenance stage."

In sharp contrast to the claimed invention, the Gupta reference does not teach or remotely suggest culturing pre-cotyledonary pine embryogenic cells on a synchronization medium before transferring the pre-cotyledonary pine embryos to the cotyledonary development medium, as claimed.

b. The Teachings of the Pullman and Gupta References Must be Considered as a Whole.

The test for obviousness is what the combined teachings of the references as a whole would have suggested to those of ordinary skill in the art. See, e.g., *In re Keller*, 642 F.2d 413, 425 (C.C.P.A. 1981); and M.P.E.P. § 2141.03. See, also, e.g., *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc.*, 230 U.S.P.Q. 416, 419 (Fed. Cir. 1986) ("It is impermissible within the framework of section 103 to pick and choose from any one reference only so much of

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it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art.").

i. The Pullman Reference

Appellants respectfully submit that the Examiner has impermissibly selected various teachings of the Pullman reference, as related to various media compositions, and combined the teachings to arrive at the synchronization medium and method of the claimed invention. It is the position of the Examiner that the singulation medium as taught by Pullman to singulate Douglas-fir proembryos is identical to the claimed synchronization medium for culturing pre-cotyledonary pine embryos. Appellants respectfully disagree for at least the reasons set forth below.

To support her position, the Examiner cites passages from Pullman that teach a singulation medium comprising gibberellin and/or abscisic acid (Col. 13, lines 40-60) and activated charcoal (Col. 13, lines 50-54), and also cites media compositions as set forth in Table 1 and Table 2 (Col. 12 to Col. 14). See Office Action, May 19, 2010, page 3.

The passages cited by the Examiner are clearly related to the culture of Douglas-fir. See, e.g., Col. 11, lines 42-44 ("the present inventors have found the basal media described in Table 1 to give excellent results, particularly when used for culturing Douglas-fir") (emphasis added); Col. 12, lines 23-25 ("[a] basal culture medium has been developed by the present inventors specifically to give more successful initiation and multiplication of Douglas-fir") (emphasis added); and Col. 13, lines 40-44 (media compositions are "advantageously used with Douglas-fir"). Furthermore, the media compositions described in Table 2 are used in Example 1, which Pullman describes as "the best mode known at present for culturing Douglas-fir by somatic embryogenesis." See Col. 13, lines 65-67 (emphasis added). It is also noted that the

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composition of the singulation medium set forth in Table 2, which Pullman describes as the best mode, does not contain activated charcoal. See Col. 13 to Col. 14, Table 2, Stage IV Singulation.

Furthermore, Pullman teaches that culturing Douglas-fir embryos requires a singulation step because Douglas-fir pro-embryos tend to form in tight clumps or clusters; however, a singulation step is not necessary for other species. See Col. 8, lines 18-21. See also, Col. 11, lines 67-68 ("the embryogeny of Douglas-fir is quite different from trees such as the spruces or pines") (emphasis added). Moreover, Pullman distinguishes the media compositions for culturing Douglas-fir from media compositions for another species, Norway spruce, by setting forth the media "particularly preferred for Norway Spruce" in Tables 7 and 8. Table 8 does not describe a singulation media. See Col. 21. Appellants note that Pullman does not specifically disclose the media compositions suitable for culturing pine embryos.

To support her position that the singulation medium for culturing pre-cotyledonary Douglas-fir embryos as taught by Pullman is equivalent to the synchronization medium for culturing pre-cotyledonary pine embryos of the claimed invention, the Examiner cites Pullman as teaching the method described can be used for many species including Loblolly pine. Col. 7, lines 50-60. See Office Action, May 19, 2010, page 3. Appellants respectfully note that the passage cited by the Examiner describes the late proembryo development culture medium, and not the singulation medium. In fact, the whole passage discusses the differences among the late proembryo development culture media compositions used to culture Douglas-fir, pine, and Norway spruce embryos. See Col. 7, line 47, to Col. 8, line 3. Pullman then describes that the culture of Douglas-fir "requires an intermediate step between the late proembryo growth stage and the final cotyledonary embryo development stage which is not necessary for other species." See Col. 8, lines 18-21.

Accordingly, it is impermissible for the Examiner to cite a passage of Pullman related to the late proembryo development medium used to culture Loblolly pine and infer that Pullman teaches a singulation medium to culture Loblolly pine, when Pullman clearly distinguishes between the late proembryo development medium and singulation medium; the composition of the late proembryo development medium is clearly different for the culture of Douglas-fir and pine; and Pullman further teaches that a singulation medium is required for the culture of Douglas-fir but is not necessary for the culture of other species.

The Examiner further cites Pullman as teaching that adding the singulation step is beneficial for improvement of proembryo quality. Col. 8, lines 5-14. See Office Action, May 19, 2010, page 7. Appellants respectfully note that the passage cited by the Examiner describes the maintenance and late proembryo development medium, and makes no reference to singulation medium. The Examiner also cites Pullman as teaching that "for virtually all coniferous species a supply of exogenous abscisic acid is a useful hormone in the development from proembryos to cotyledonary embryos . . . this was always used in combination with an absorbent such as activated charcoal." Col. 9, lines 49-55. See Office Action, May 19, 2010, page 7. Again, it is clear that the quoted passage describes a development media and not a singulation media. The Examiner further adds that Pullman teaches the combination of abscisic acid with gibberellins reduces the tendency to precocious germination. See Office Action, May 19, 2010, page 7. Appellants note that germination is a step in the embryogenesis process that occurs after embryo development. See, e.g., Specification, page 1, lines 19-22.

Appellants respectfully submit that the Examiner has impermissibly taken teachings of Pullman related to maintenance and/or development media to support her position that the singulation step of Pullman is identical to the synchronization step of the claimed invention. As discussed supra, Pullman clearly teaches different media compositions for different stages in the

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process and for different species. See, e.g., Col. 13 to Col. 14, Table 2, which sets forth the media compositions for culturing Douglas-fir (Stage I, Initiation; Stage II Maintenance 1; Stage III Maintenance 2; Stage IV Singulation; Stage V Development; and Stage VI Germination); see also, Col. 21, Table 8, which sets forth the media compositions for culturing Norway spruce (BM_I - Induction Medium; BM_M - Maintenance Medium; BM_D - Development Medium; BM_G - Germination Medium). As discussed supra, the synchronization medium of the claimed method is used for the culture of pre-cotyledonary pine embryogenic cells in an intermediate synchronization step that occurs after the maintenance step and before the development step. Therefore, there is no basis for the Examiner to assert that the teachings of Pullman as regards maintenance and/or development medium are relevant to the claimed synchronization medium for the culture of pine.

Accordingly, the Examiner has not taken the teachings of the Pullman reference as a whole, and has selectively cited the teachings of Pullman related to maintenance and/or development medium and impermissibly characterized those teachings as related to the claimed synchronization medium.

ii. <u>The Gupta Reference</u>

The Examiner cites Gupta as teaching a singulation medium comprising maltose as the principal source of sugar. See Office Action dated May 19, 2010, page 4. Appellants submit that this teaching of Gupta is irrelevant to the claimed invention. Similar to Pullman, Gupta describes a singulation step for culturing <u>Douglas-fir</u> embryos. See Col. 8, lines 20-23 ("Maltose has again been found, very advantageous in place of sucrose as the carbon and energy source in Douglas-fir singulation media.") Gupta also states that "the embryogeny of Douglas-fir is quite different from trees such as the spruces or pines." See Col. 12, lines 24-25.

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Appellants note that Gupta sets forth the "preferred " media compositions for Douglas-fir in Tables 1 and 2 (see Col. 12, lines 50-51; see also Col. 13 to Col. 14, Tables 1 and 2); for Norway spruce in Tables 11 and 12 (see Col. 21, line 49, to Col. 22, line 58); and for Loblolly pine in Tables 13 and 14 (see Col. 23, line 1, to Col. 24, line 10). Although Table 2 sets forth the composition of singulation medium for the culture of Douglas-fir, Table 12, which describes the media compositions for culture of Norway spruce, and more importantly, Table 14, which describes the media compositions for culture of Loblolly pine, make no mention of a singulation medium. Accordingly, it is impermissible for the Examiner to apply a teaching of Gupta as relates to the culture of Douglas-fir embryos to arrive at the claimed invention for the culture of pine embryos.

c. All Elements of the Claimed Invention Are Not Taught by the Pullman and Gupta References.

In order to establish a prima facie case of obviousness, all of the claimed elements must be found in the prior art. See M.P.E.P. § 2143.

As discussed supra, Claim 1 includes at least the following elements: (a) cultivating pre-cotyledonary pine embryogenic cells in, or on a maintenance medium; (b) cultivating pre-cotyledonary pine embryogenic cells from step (a) for a period from one week to two weeks in, or on, a synchronization medium comprising maltose as the principal metabolizable sugar source, an absorbent composition and at least one synchronization agent selected from the group consisting of abscisic acid and a gibberellin, wherein the absorbent composition and the at least one synchronization agent are present at a concentration effective to produce a synchronized population of pre-cotyledonary pine somatic embryos wherein at least 50% of the pre-cotyledonary pine somatic embryos in the synchronized population are at the same developmental stage; and (c) transferring the synchronized population of pre-cotyledonary pine

somatic embryos from step (b) to a development medium and culturing the pre-cotyledonary pine somatic embryos to produce a synchronized population of cotyledonary pine somatic embryos. The claimed invention comprises a synchronization step between the maintenance step and the development step.

As discussed in detail supra, the Pullman and Gupta references, alone or in combination, do not teach or remotely suggest a synchronization step for the culture of pre-cotyledonary <u>pine</u> somatic embryos, as claimed. Pullman provides no teaching regarding a synchronization step in the culture of pine embryos. Gupta describes the following steps for the culture of Loblolly pine: Stage I—Induction; Stage II—Maintenance; Stage III—Embryo Development; Stage IV—Germination; and Stage V—Conversion. Gupta clearly does <u>not</u> teach a synchronization step for the culture of <u>pine</u> embryos. See Col. 23, line 1, to Col. 25, line 35.

Accordingly, because the Pullman and Gupta references, alone or in combination do not teach or remotely suggest a <u>synchronization</u> step in which pre-cotyledonary <u>pine</u> embryogenic cells are cultured in, or on, a synchronization medium to produce a synchronized population of pre-cotyledonary pine somatic embryos, as claimed, the Pullman and Gupta references do not teach all the elements of Claim 1.

d. <u>There is no Motivation to Modify the Pullman and Gupta References to Arrive at the Claimed Invention.</u>

To support a finding of obviousness, there must be some articulated reason with some rational underpinnings that one skilled in the art would modify the references to arrive at the claimed invention. See M.P.E.P. § 2143.01(IV). It is the position of the Examiner that the synchronization medium for culturing pre-cotyledonary pine embryos in the claimed method is equivalent to the singulation medium for culturing Douglas-fir proembryos. Appellants respectfully disagree. As discussed supra, both Pullman and Gupta teach a singulation step in the

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context of culturing Douglas-fir proembryos because of the tendency of Douglas-fir proembryos to form tight clumps or clusters. Neither the Pullman nor Gupta references teach a singulation step for the culture of pine pre-cotyledonary embryos. In fact, Pullman states, "Douglas-fir generally requires an intermediate step between the late proembryo growth stage and the final cotyledonary embryos development stage which is not required for other species." See Col. 8, lines 18-21 (emphasis added). Accordingly, one of skill in the art, in view of the Pullman and Gupta references, would not be motivated to add a singulation step for the culture of pine pre-cotyledonary embryos.

Furthermore, assuming arguendo, that one skilled in the art would be motivated to add an intermediate step between the maintenance stage and the development stage for the culture of pre-cotyledonary pine embryos, appellants submit that one skilled in the art would not be motivated, in view of the Pullman and Gupta references, to add an absorbent composition to the singulation medium as taught by Pullman and Gupta to arrive at the claimed synchronization medium. Pullman describes the examples as the "best mode known at present for culturing Douglas-fir by somatic embryogenesis." See Col. 13, lines 65-67 (emphasis added). The composition of the singulation medium used in the examples in Pullman is set forth in Table 2, Col. 13 to Col. 14. It is clear from the information in Table 2 that the singulation medium does not contain an absorbent composition. Similarly, Gupta describes the examples as the "best mode known at present for culturing Douglas-fir by somatic embryogenesis." See Col. 14, lines 22-24 (emphasis added). The composition of the singulation medium used in the examples is set forth in Table 2, Col. 13 to Col. 14. It is clear from the information in Table 2 that the singulation medium does not contain an absorbent composition.

Accordingly, because both Pullman and Gupta describe the singulation medium set forth in Table 2 of each reference as the "best mode" and the best mode singulation medium does not

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contain an absorbent composition, there is no motivation for one skilled in the art to waste time and resources in altering the best mode as described by Pullman and Gupta by adding an absorbent composition to arrive at the claimed synchronization medium.

e. The Claimed Invention Achieves Unexpected Results.

Non-obviousness can be shown by providing evidence that the claimed invention yields unexpectedly improved properties or properties not present in the prior art. See M.P.E.P. § 2145. As further evidence of non-obviousness, appellants note that the claimed method produces unexpected results. Appellants have surprisingly found that adding an intermediate synchronization step in the culture of pine embryos between the maintenance step and the cotyledonary development step, and culturing pre-cotyledonary pine embryogenic cells on a synchronization medium that comprises an absorbent composition and at least one synchronization agent selected from the group consisting of abscisic acid and a gibberellin, for a period of from one week to two weeks results in a synchronized population of pre-cotyledonary pine embryos that are relatively uniform in size, shape, and stage of development.

As discussed supra, the Pullman and Gupta references teach, as the best mode, a singulation medium for the culture of Douglas-fir that contains abscisic acid and gibberellins, but does not contain an absorbent composition.

In sharp contrast to the singulation medium as described in Pullman and Gupta for the culture of Douglas-fir, appellants have found that a combination of abscisic acid and/or gibberellins, and an absorbent composition is necessary to produce a synchronized population of pre-cotyledonary pine embryos, as shown below.

The addition of activated charcoal alone (synchronization medium 1) resulted in precocious embryo development. The addition of abscisic acid alone (synchronization medium 2) produced embryos in many different stages of development, as did the addition of

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gibberellins alone (synchronization mediums 4 and 5). However, the addition of activated charcoal in combination with abscisic acid (synchronization medium 3); the addition of activated charcoal in combination with gibberellins (synchronization medium 6); and the addition of activated charcoal in combination with abscisic acid and gibberellins (synchronization medium 7) inhibited precocious embryo development and greening, and resulted in embryos that were more uniform in size.

Nothing in the prior art of record even remotely addresses the unexpected result obtained by the claimed invention.

f. Conclusion

Claim 1 is patentable over the Pullman reference in view of the Gupta reference because the Examiner has not considered the teachings of the Pullman and Gupta references as a whole; the Pullman and Gupta references, either alone or in combination, do not teach all the elements of Claim 1; there is no motivation to modify the Pullman and Gupta references to arrive at the claimed invention; and the claimed invention achieves unexpected results. Accordingly, the Examiner has not established a *prima facie* case of obviousness and Claim 1 is non-obvious and patentable over the Pullman reference in view of the Gupta reference. Appellants respectfully request reversal of this ground of rejection.

2. Claims 2-13, 17-19, 21, and 23-26

Claims 2-13, 17-19, 21, and 23-26 depend from Claim 1. If an independent claim is nonobvious under 35 U.S.C. § 103, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071 (Fed. Cir. 1988); see also M.P.E.P. § 2143.03. Accordingly, Claims 2-13, 17-19, 21, and 23-26 are non-obvious and patentable over the Pullman reference in view of the Gupta reference. Appellants respectfully request reversal of this ground of rejection.

VIII. CLAIMS APPENDIX

1. A method for producing a synchronized population of pine somatic embryos, the method comprising:

(a) cultivating pre-cotyledonary pine embryogenic cells in, or on a maintenance medium comprising nutrients that sustain the pine embryogenic cells;

(b) cultivating pre-cotyledonary pine embryogenic cells from step (a) for a period from one week to two weeks in, or on, a synchronization medium that comprises maltose as the principal metabolizable sugar source, an absorbent composition and at least one synchronization agent selected from the group consisting of abscisic acid and a gibberellin, wherein the absorbent composition and the at least one synchronization agent are present at a concentration effective to produce a synchronized population of pre-cotyledonary pine somatic embryos wherein at least 50% of the pre-cotyledonary pine somatic embryos in the synchronized population are at the same developmental stage; and

(c) transferring the synchronized population of pre-cotyledonary pine somatic embryos from step (b) to a development medium and culturing the pre-cotyledonary pine somatic embryos for a period from 9 to 14 weeks to produce a synchronized population of cotyledonary pine somatic embryos.

2. The method of Claim 1 wherein the absorbent composition is selected from the group consisting of activated charcoal, soluble poly(vinyl pyrrolidone), insoluble poly(vinyl pyrrolidone), activated alumina, and silica gel.

3. The method of Claim 2 wherein the absorbent composition is activated charcoal.

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4. The method of Claim 1 wherein the concentration of the absorbent composition in the synchronization medium is from about 0.5 g/L to about 50 g/L.

5. The method of Claim 1 wherein the absorbent composition is activated charcoal, and the activated charcoal is present in the synchronization medium at a concentration in the range of from about 0.1 g/L to about 5 g/L.

6. The method of Claim 1 wherein the absorbent composition is activated charcoal, and the activated charcoal is present in the synchronization medium at a concentration in the range of from about 0.5 g/L to about 1 g/L.

7. The method of Claim 1, wherein abscisic acid is used as a synchronization agent.

8. The method of Claim 1, wherein a gibberellin is used as a synchronization agent.

9. The method of Claim 1, wherein abscisic acid and at least one gibberellin are used as synchronization agents.

10. The method of Claim 1, wherein a gibberellin is present in the synchronization medium at a concentration of from about 0.5 mg/L to about 500 mg/L.

11. The method of Claim 1, wherein a gibberellin is present in the synchronization medium at a concentration of from about 1.0 mg/L to about 100 mg/L.

12. The method of Claim 1, wherein abscisic acid is present in the synchronization medium at a concentration of from about 1.0 mg/L to about 500 mg/L.

13. The method of Claim 1, wherein abscisic acid is present in the synchronization medium at a concentration of from about 0.5 mg/L to about 20 mg/L.

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17. The method of Claim 1, wherein the osmolality of the synchronization medium is from about 90 mM/Kg to about 300 mM/Kg.

18. The method of Claim 1, wherein the pH of the synchronization medium is from about 5 to about 6.

19. The method of Claim 1, wherein Loblolly pine somatic embryos are produced from Loblolly pine embryogenic cells.

21. The method of Claim 1, wherein at least 75% of the embryos in the synchronized population of pine somatic embryos are at the same developmental stage.

23. The method of Claim 1, wherein the osmolality of the development media of step (c) is higher than the osmolality of the synchronization media of step (b).

24. The method of Claim 1, wherein the osmolality of the synchronization media of step (b) is from about 90 mM/Kg to about 300 mM/Kg; and the osmolality of the development media of step (c) is from about 250 mM/Kg to about 450 mM/Kg.

25. The method of Claim 1, wherein the synchronization medium of step (b) is a solid medium.

26. The method of Claim 1, wherein the synchronization medium of step (b) is a liquid medium.

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IX. EVIDENCE APPENDIX

None.

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X. RELATED PROCEEDINGS APPENDIX

None.

Respectfully submitted,

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